Non heat Shock Transformation

RBC HIT Competent Cell Protocol Book

Product Description

Cat.No.	Items	Strain	Spec
RH617-J	JUMBO HIT-DH5α	DH5α	1 ml x 2
RH617	Value 10^8 HIT-DH5α	DH5α	100 µl x 10
RH618	High 10^8 HIT-DH5α	DH5α	100 µl x 10
RH619	Super 10^9 HIT-DH5α	DH5α	100 µl x 10
RH717-J	JUMBO HIT-JM109	JM109	1 ml x 2
RH717	Value 10^8 HIT-JM109	JM109	100 µl x 10
RH718	High 10^8 HIT-JM109	JM109	100 µl x 10
RH117-J	JUMBO HIT-Blue	Xl-Blue	1 ml x 2
RH117	Value 10^8 HIT-Blue	Xl-Blue	100 µl x 10
RH118	High 10^8 HIT-Blue	Xl-Blue	100 µl x 10
RH119	Super 10^9 HIT-Blue	Xl-Blue	100 µl x 10
RH217	HIT-21	Bl21 (DE3)	100 µl x 5
RG001 (autoclaved)	HIT Plating Beads		100 g, 4 mm

Competent cells must be stored in a stable -70°C to -80°C refrigerator.

Notes

blue/white screening, general cloning -> $5X10^7$ transformants/µg pUC19
blue/white screening, general cloning -> $5X10^7$ transformants/µg pUC19
blue/white screening for generation of cDNA libraries and subcloning -> $1X10^8$ transformants/µg pUC19
blue/white screening for generation of cDNA libraries and subcloning -> $5X10^8$ transformants/µg pUC19
8-10 hours growth, blue/white screening, robotic screening, general cloning -> $5X10^7$ transformants/µg pUC19
8-10 hours growth, blue/white screening, robotic screening, general cloning -> $5X10^7$ transformants/µg pUC19
8-10 hours growth, blue/white screening, robotic screening, general cloning -> $1X10^8$ transformants/µg pUC19
general cloning, blue/white screening -> $5X10^7$ transformants/µg pUC19
general cloning, blue/white screening -> $5X10^7$ transformants/µg pUC19
general cloning, blue/white screening, libraries -> $1X10^8$ transformants/µg pUC19
general cloning, blue/white screening, libraries -> $5X10^8$ transformants/µg pUC19
general cloning Protein expression -> $5X10^6$ transformants/µg pUC19
spread competent cells, 75~100 plates

Fastest worldwide: 1 min protocol

RBC HIT E. coli competent cells provide the fostest true single-step transformation process world-wide (1 tube, from transformation to plating)

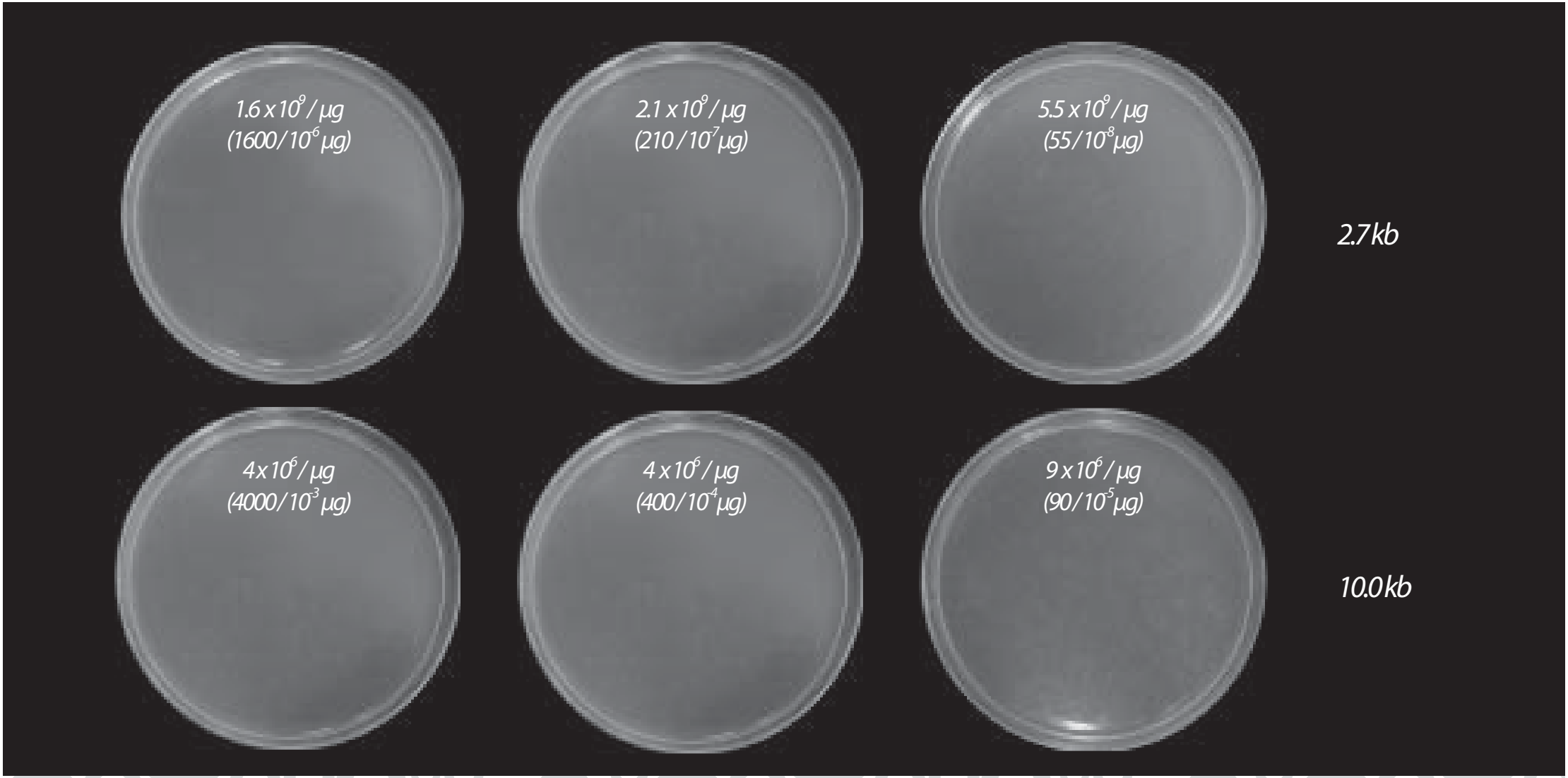
High efficiency:

10^7-10^9 transformants/μg pUC19 plasmid
E. coli DNA transformation effciency reaches 10^7~10^9 transformants/μg pUC19 plasmid DNA (varies according to strains and plasmid size)

Calculation of transformation efficiency

Method	HIT Non-Heat Shock Protocol
Formula	transformation efficiency = (transformed colonies) / (μg of plasmid)
Example	5.5×10^9/μg(efficiency) = 55 (transformed colonies) / 10^{-8} μg
Test for	RH619 : Super 10^9 HIT-DH5α
Selection	LB agar (Ap50 μg/ml)
Results	test with 10^6~10^8 μg 2.7 kb plasmid, resulted in efficiency of 1.6~5.5×10^9/μg test with 10^{-3}~10^{-5} μg 10.0 kb plasmid, resulted in efficiency of 4.0~9.0×10^6/μg

Plasmid Size



Contents and Notes

1 **JUMBO HIT: 10^{7-8} Efficiency (Requires Freeze-Thaw aliquoting)**

2 vials of 1 ml JUMBO HIT competent cells (should be stored at -70˚C ~-80˚C)

QC report and Control plasmid (pUC19, 10^{-4} µg/µl, stored at -20˚C ~-70˚C)

2 **Value 10^{8} : 10^{7-8} Efficiency (Ready to Use)**

10 vials of 100 µl value 10^{8} HIT competent cells (should be stored at -70˚C ~-80˚C)

QC report and Control plasmid (pUC19, 10^{-4} µg/µl, stored at -20˚C ~-70˚C)

3 **High 10^{8} HIT: 10^{8} Efficiency (Ready to Use)**

10 vials of 100 µl high 10^{8} HIT competent cells (should be stored at -70˚C ~-80˚C)

QC report and Control plasmid (pUC19, 10^{-4} µg/µl, stored at -20˚C ~-70˚C)

4 **Super 10^{9} HIT: 10^{9} Efficiency (Ready to Use)**

10 vials of 100 µl Super 10^{9} HIT competent cells (should be stored at -70˚C ~-80˚C)

QC report and Control plasmid (pUC19, 10^{-4} µg/µl, stored at -20˚C ~-70˚C)

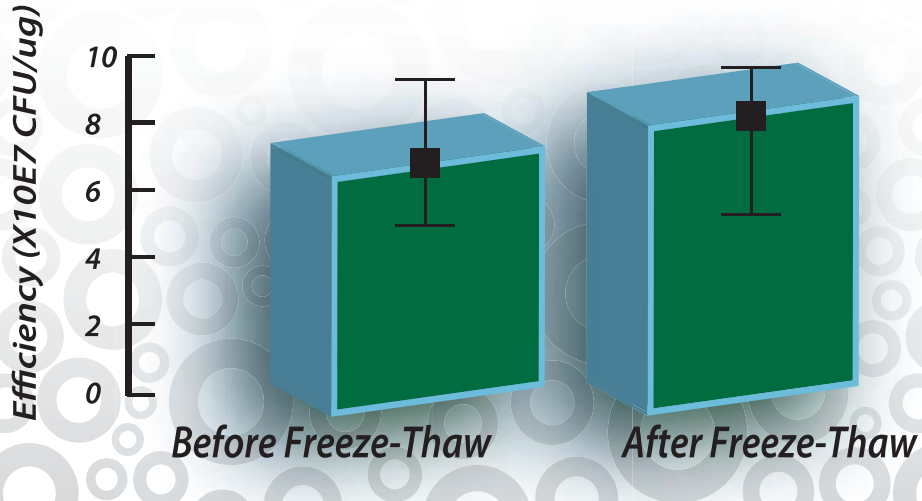
Go online to **WWW.RBCBIOSCIENCE.COM** to search the full ranae of RBC Bioscience research products

Expiry date: 1 year from date of manufacture

Notes for dispensing JUMBO HIT competent cells

JUMBO HIT may be dispensed in aliquots and refrozen with extremely high efficiency (90-100%). The whole procedure must be completed within 5 min. Use running tap water or water bath to fast thaw competent cells to 1/3-volume thawed state (10-20 sec.)- incubate on ice until almost fully thawed (10-20 sec.)– dispense into 100 µl aliquots on ice and immediately refrigerate at -70˚C~-80˚C.

Effect of Freeze-Thaw



Contents and Notes

Genotypes	Applications
end A	*Prevents plasmid degradation during extraction*
recA	*Prevents DNA recombination*
hsd	*Enhances transformation efficiency of selected PCR DNA strands and cDNA libraries*
deoR	*Enhances transformation efficiency of high MW plasmids and cosmids*
LacZ M15	*Blue-White screening*
Lon	*Lon Protease Deficient, Improves Protein Yield*
ompT	*OmpT Protease Deficient, Improves Protein Yield*

	HIT-DH5α	HIT-JM109	HIT-Blue	HIT-21
	F-(80d lacZ M15) (lacZYA-argF)U169 hsdR17(r- m+) recA1 endA1 relA1 deoR	F'traD36 proA+proB+ laclq (lacZ)M15 l (lac-proAB) hsdR17 recA1 endA1 relA1	hsdR17(rk- mk+), recA1, endA1, gyrA96, thi-1,s upE44, relA1, lac[F' proAB laclqZDM15Tn10(Tet')]	E.coli B, F-, dcm, ompT, hsdS(rB-mB-), gal (DE3)
	Yes	Yes	Yes	No
	Yes	Yes	Yes	No
	Yes	Yes	Yes	Yes
	Yes	No	No	No
	Yes	Yes	Yes	No
	No	No	No	Yes
	No	No	No	Yes

HIT Non-Heat Shock Transformation Protocol

(1~10 minutes, efficiency = 10^7 ~10^9 /μg)

Attention : prior to transformation, dry plating beads and agar plates should be warmed to 37ºC (strongly recommended)

Prepare ice bucket, 37°C plating beads and selective plates. Thaw competent cell vial with room-temp. tap water or water bath for 10~20 seconds until 1/3 thawed.

▼

Add DNA whose volume is less than 10% of volume of cells . Vortex 1 second.

▼

Place on ice for 1-10 minutes

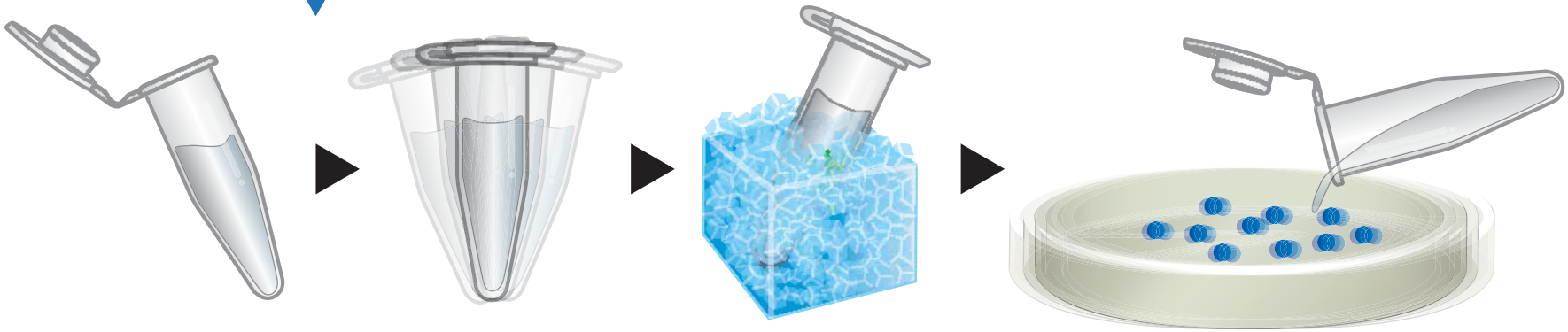
▼

Plate Transfer onto 37°C dry selection plate media, spread using RBC plating beads.

▼

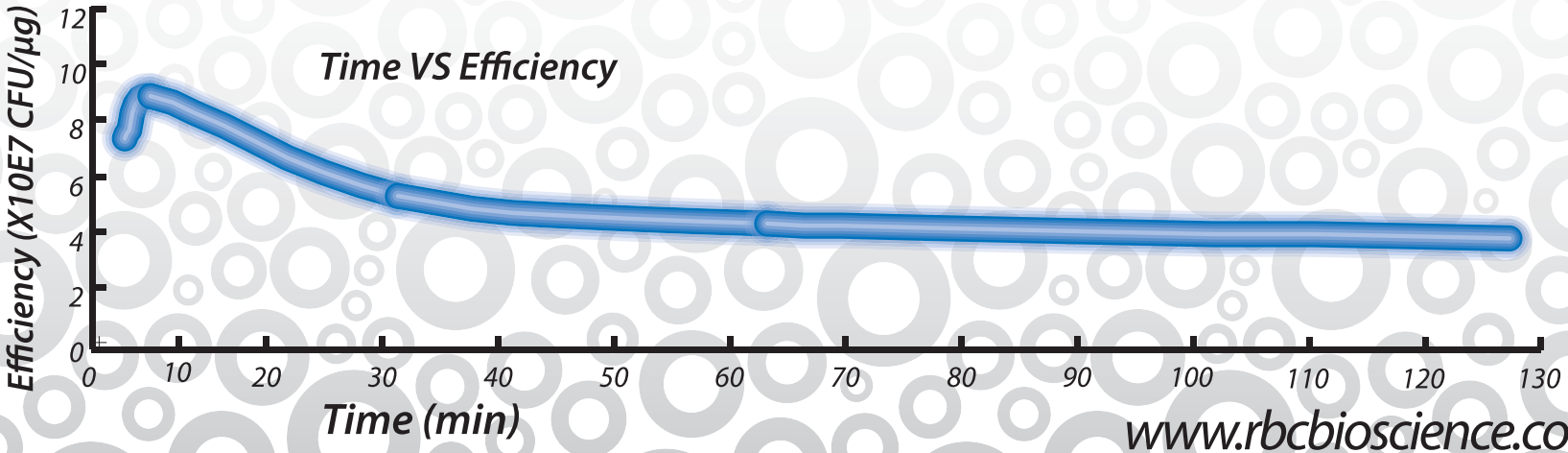
Immediately incubate plate at 37°C (16-18 hours for HIT-DH5α, 8-24 hours for HIT-109).Observe growth of transformed colonies.

Vector



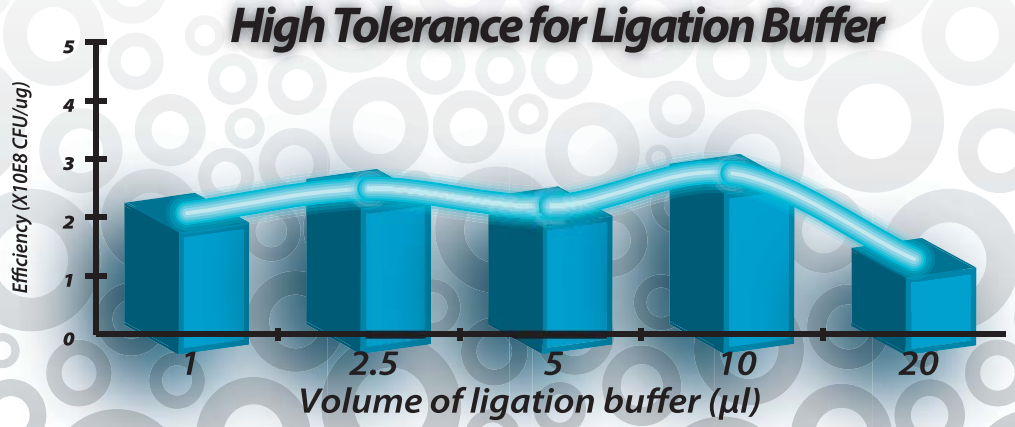
Rapid Thaw | Vortex 1 ssec | 0°C Incubate on Ice | Plate

10^7 to 10^9 Efficiency : High Ice Transformation



Notes

1. *RBC HIT Competent cells provide best efficiency when cells are about l/3-volume thawed state (cells in completely thawed state will cause a~ 3 fold decrease in transformation efficiency).*
2. *Vortexing for 1 second will not affect the efficiency (HIT cells can withsand high speed vortex)*
3. *Modifid protocol for large plasmids (>6 kb) and cDNA libraries : 20-min ice bath followed by 1- min 42˚C water bath and another 20-min ice bath. Efficiency will increase 2-5 fold.*
4. *Further incubation with either SOC or LB medium is not required.*
5. *Plating using plating beads 37˚C and selective plates improves the transformation efficiency up to 3 fold when compared with room temperlg ture plating beads.*
6. *The antibiotic concentrations are recommended as: Ap: 50 µg/ml; Km: 30 µg/ml, Tc: 12.5 µg/ml for HIT DH5α and HIT-JM 109 Strains. Higher antibiotic concentrations will decrease the efficiency. Lower conc. will increase the number of satellite colonies.*
7. *HIT DH5α and HIT- JM 109: Warning- over incubation σt 37˚C for18-24 hours will result in satellite (pseudo-positive) colonies appeσring.*



Q&A

I can't see any colonies on my plate, is there something wrong with my HIT Competent cells?

HIT Competent cells are batch tested at manufacture. They are also temperature controlled AT ALL TIMES during shipping and storage. ALWAYS USE THE CONTROL PLASMID supplied to provide a reference transformation experiment. If the control DNA is not transforming HIT and you are sure the cells have been stored correctly AND you have followed the correct protocol, then it's time to contact your distributor or RBC Bioscience.

What are the major differences between HIT Competent cells strains ?

HIT Competent cells strains are common public lab strains. For reference strain and genotype check individual listing. HIT-DH5α is a strain which has been engineered for cloning large plasmids and library construction. HIT-JM109 is a strain that grows faster and is excellent in blue/white and robotic screening. HIT-Blue is also popular for regular cloning and Blue/White screening. HIT-21 is ideal for protein expression.

Can I transform directly with my ligation product?

It depends on the efficiency required. If you require high efficiency you should always remove any ligase enzyme, PEG or detergent that may be present. These are known inhibitors of transformation. Recommended clean up methods are RBC's HiYield Gel/PCR Extraction Kit or precipitate the DNA directly. Resuspend in TE or distilled water. Checking your ligation product on an agarose gel is always a good idea, both for extraction purity and ligation. For regular cloning, it is usually sufficient to dilute the ligation solution three fold and add 1ul. Do NOT use more than 10ng total DNA/transformation, otherwise your tranformation efficiency will start to drop sharply. Do not add more than 10% volume of ligation products to total volume HIT competent cells.

Can HIT competent cells be freeze-thawed repeatedly?

Extensive freeze-thaw testing indicates HIT competent cells can be thawed, dispensed in aliquots and refrozen while maintaining 90~100% efficiency if completed within 5 min. Use running water or water bath to rapidly thaw competent cells to about 1/3-volume thawed state (10-20 sec.). Incubate on ice until fully thawed (10-20 sec.) and immediately dispense on ice. Store cells at -70°C. Maximum three times freeze thaw.

Does the storage temperature and thawing method affect competent cells' transformation efficiency?

HIT competent cells should be stored at -70°C~-80°C condition. Slow thawing caused by power outages and unstable freezers will result in decreased efficiency.

Is there a difference between using plating beads and plating loop in terms of the transformation efficiency?

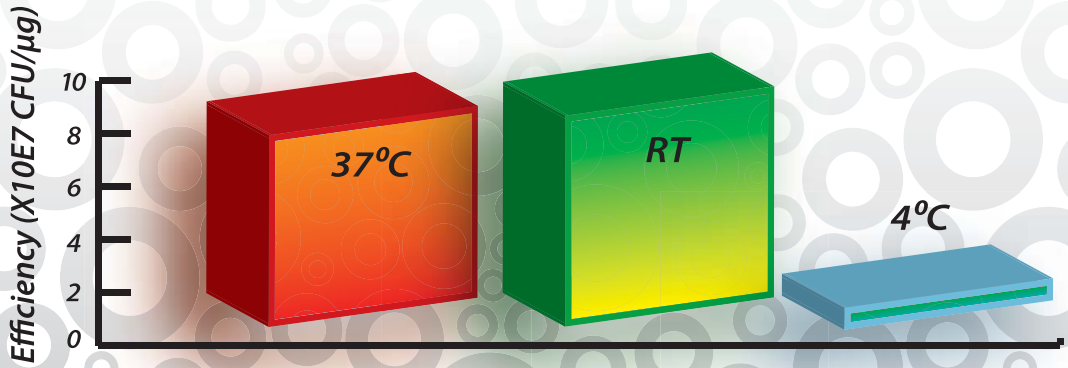
Plating beads result in significantly higher transformation efficiencies than seen using a plating loop, probably due to increased surface spreading.

Do temperature and condensation of plating beads or plates affect transformation efficiency?

Use plating beads and plates at 37°C to greatly increase transformation efficiency. In addition, compared with wet plating beads and plates, dried plating beads and plates give increased transformation efficiency and lower numbers of satellite colonies.

How to make dry and warm selection plates?

After pouring plates, uncover the plates in a laminar flow hood, evaporate for 30-60 min, cover each plate and warm in incubator prior to transformation at 37°C.



For the transformation of larger plasmids, is it necessary to change the standard transformation procedure?

For the transformation of plasmids with higher molecular weight or cDNA libraries (vector + insert >6 kb), the standard procedure may be modified to a 20 min ice bath -1 min 42℃ Heat Shock - 20 min ice bath protocol to increase transformation efficiency.

What is the optimum incubation time on ice?

Between 1-10 minute ice incubation will have no significant difference. Over ten minutes incubation will result in decreased efficiency.

What are the major differences between HIT-DH5α,HIT-JM109, HIT-Blue and HIT-21?

Please refer to product table and genotype table on pg H-2.

Does the concentration of ampicillin in the selection medium affect transformation efficiency?

For HIT-DH5α: LB + Ap 50-60 μg/μl results in 2~3 times more transformation efficiency than LB + Ap 100 μg/μl. Transformed colonies can be observed after 11~16 hours cultivation, but after 18 hours satellite populations will appear around positive colonies.For HIT-JM109: LB + Ap 50-100 μg/μl brings similar transformation efficiencies. Transformed colonies can be observed after 8~10 hours cultivation, but after 24 hours satellite colonies around positive colonies will also form.

Does the size of plasmid affect transformation efficiency?

Transformation efficiency = the numbers of transformed colonies/ mass of plasmids (μg). For instance, Super 109 competent cells can reach 1.6~5.5 x 109/μg with 2.7-kb plasmids, but only 4.0~9.0 x 106/ μg with 10.0-kb plasmids.

How do I reduce interference of satellite colonies?

1. *Use warm and dry plating beads and plates.*
2. *Please check and test the antibiotics, since the efficacy of antibiotics may be influenced by the method of preparation, different batches, sources and expiration dates.*


RBCBioscience